DIFFERENT RATES OF SPONTANEOUS MUTATION DURING MITOSIS AND MEIOSIS IN YEAST

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SPONTANEOUS mutability has been studied principally in systems organized in biologically distinct ways: in microorganisms, where mutations are recognized after mitosis or paramitosis, and in higher organisms, where the mutations that have been studied are mostly meiotic in origin.

Data collected in microorganisms permit analyses of mutation rates in terms of mutations per cell per division (LURIA and DELBRÜCK 1943; RYAN and WAINWRIGHT 1954) or mutations per cell per unit time (NOVICK and SZILARD 1951; NOVICK 1955). In higher organisms the spontaneous mutation frequencies are not difficult to obtain, but the spontaneous mutation rates are very difficult to quantitate. Mitosis and meiosis are systems in which the chromosome behavior and internal environment are different, and therefore a comparative study of these two systems could lead to a better understanding of the mechanisms of origin of spontaneous mutations. Of course, this would only be true if differences were found. There is some evidence that spontaneous mutation frequency in Drosophila is higher during gametogenesis than during the gonial stages (MULLER 1958). This conclusion is based on the relatively low frequency of clusters of visible mutations from single individuals (SCHALET 1958), but a quantitative analysis on a per cell basis would be desirable.

Mutations in yeast can be treated quantitatively on a per cell basis during either the vegetative (mitotic) or sporulating (meiotic) stages of its life cycle. Therefore, we undertook a series of experiments to determine in a clear-cut manner if suspected differences in spontaneous mutation rates were real.

In this study we analyzed reversions to wild type of mutants for biochemical deficiencies. These will be referred to as revertants rather than as back mutants since a back mutation implies considerably more knowledge of intragenic site specificity than the data warrant.

MATERIALS AND METHODS

Culture media

(a) *Y. E. medium 21:* The medium has all the growth requirements for yeast present: Costantino yeast autolysate, 3 g; Costantino casein hydrolysate, 3 g;

KH_2PO_4, 1.5 g; $MgSO_4$, 0.5 g; $(NH_4)_2SO_4$, 2 g; $CaCl_2$, 0.32 g; KI, 100 μg; boric acid, $MnSO_4$, and ammonium molybdate, 10 μg; $FeSO_4$, 50 μg; thiamine, riboflavine, pyridoxine, nicotinic acid, PABA, and calcium pantothenate, 200 μg; Inositol, 10 mg; Biotin, 2 μg; glucose, 20 g; distilled water, 1000 ml; pH 5.6–5.7; three percent agar was added when solid medium was required.

(b) *Minimal medium 40:* This medium was used for selecting revertants: KH_2PO_4, 1g; $MgSO_4$, 0.5 g; NaCl, 0.1 g; $CaCl_2$, 0.1 g; $(NH_4)_2SO_4$, 3.5 g; oligominerals and vitamins as in medium 21; glucose, 10 g; distilled water, 1000 ml; pH 5.6–5.7. In addition to the marker selected for reversion, in all cases, either hemizygous (haploid strains) or heterozygous (diploid strains) biochemical markers were introduced in our stocks. Minimal medium was supplemented with 30 μg/ml (500 μg/ml for threonine) of the amino acids required by these extra markers.

(c) *Sporulation medium 23:* This is composed of sodium acetate, 5 g; 1000 ml distilled water; pH 6.8. It was used in liquid form under aeration in continuously shaken tubes. All diploid strains used were high frequency sporulators. Sporulation ranged from 45 to 85 percent in all our experiments, and no correlation was found between mutation rate and spore formation frequencies.

Removal of the ascus wall

In order to avoid competition in spore germination in packed asci, revertant frequency in sporulated culture was always tested on spores after removal of the ascus wall by treating sporulated cultures with *Bacillus polymyxa* enzyme (BPE). This catalytic agent has been described by WRIGHT and LEDERBERG (1957) and is now routinely used in our laboratory.

(a) *Preparation:* Approximately 10^7 cells of *B. polymyxa* strain BP 87, derived from the original strain C7 and selected for higher production of lysing activity, were grown on slants of PS agar with one percent glucose added (Costantino peptone, 6 g; Costantino yeast autolysate, 3 g; Costantino beef extract, 1.5 g; Costantino casein hydrolysate, 4 g; NaCl, 5 g; H_2O, 1000 ml; pH 7.3–7.4; agar when required, 20 g). Cells were transferred into 500 ml shaker flasks containing 100 ml of PS medium without glucose, with 10^7 cells/ml of freshly sporulated yeasts added (BPE is adaptive) and shaken at 28°C for five days. Under these conditions the bacteria grow well and produce the extracellular lytic principle, while yeast spores do not germinate. The grown culture was centrifuged and the clear supernatant precipitated by saturation with ammonium sulfate. The precipitate and the foam formed during precipitation were dried for two days under vacuum at room temperature. The powder obtained can be stored on silica gel at 4°C up to two to three months without losing activity.

(b) *Ascus wall lysis:* This is obtained by adding ten to 300 μg of BPE dried extract (depending on the specific activity of the preparation) to one ml of a distilled water suspension (10^8 cells per ml) of freshly sporulated yeast culture in tap water and shaking in tubes at 28°C for 24 to 36 hours. Ascus walls are completely lysed without damage to the spores or to unsporulated cells; both show 100 per cent survival even after five days of treatment.

Strains

The following strains of *Saccharomyces cerevisiae* were used:

Haploid strain: 10102 $a, hi_1\, Tr_2, met_2$

Diploid strains: 5160 $\dfrac{a}{\alpha}, \dfrac{hi_1}{Hi_1}\, \dfrac{Tr_2}{tr_2}, \boxed{\dfrac{met_2}{met_2}}$

5207 $\dfrac{a}{\alpha}, \dfrac{Ur_3}{ur_3}, \dfrac{hi_3}{Hi_3}, \boxed{\dfrac{ar_8}{ar_8}}$

5209 $\dfrac{a}{\alpha}, \dfrac{ur_3}{Ur_3}\, \dfrac{thr_3}{Thr_3}\, \boxed{\dfrac{hi_1}{hi_1}}\, \dfrac{ar_6}{Ar_6}\, \dfrac{is_1}{Is_1}\, \dfrac{tr_2}{Tr_2}, \dfrac{ad_1}{Ad_1}, \dfrac{Ly_2}{ly_2}$

The strain numbers are those of the Pavia collection. The diploid strains homozygous for any mutant (enclosed in boxes) were prepared by introducing the two mutant genes into the diploid starting from the same parent haploid. These strains are, therefore, carrying strictly homoallelic (identical) mutant genes on both chromosomes. The following abbreviations are used here: a and α = mating type alleles, ad = adenine dependence, ar = arginine dependence, is = isoleucine dependence, met = methionine dependence, thr = threonine dependence, tr = tryptophan dependence. We have adopted HAWTHORNE and MORTIMER's (1960) marker enumeration. Mutants ad_1, met_2, and ar_8 have been produced in our laboratory by UV irradiation and classified by complementation tests with HAWTHORNE and MORTIMER's standard strains.

Genetic analysis

All crosses and segregation analyses were carried out according to standard procedures. Copulations were always performed between haploid strains carrying different markers, and individual zygotes were selected on minimal medium. Diploids were sporulated in medium 23, ascus walls dissolved with BPE, and spores isolated by micromanipulation.

METHODS OF ESTIMATING SPONTANEOUS MUTATION RATES

The life cycle of *S. cerevisiae* is too well known to describe in detail here. In our experiments diploid cells grown through the vegetative cycle (mitosis) in YE presporulation medium 21 undergo sporulation (meiosis) when transferred to sporulation medium 23. The revertant frequencies from which the mutation rate was computed have been estimated by sampling the vegetative cultures in the logarithmic phase of growth and by sampling from sporulated cultures after one meiotic duplication. The mutations studied in this work are reversions from auxotrophy (recessive) to prototrophy (dominant) in diploid cells carrying the specific biochemical requirement in the homozygous state. Specifically, estimates of phenotypic reversion frequency have been performed in strain 5160 for *met* to *Met*, in strain 5207 for *ar* to *Ar*, and strain 5209 for *hi* to *Hi*.

Mitosis

General procedure: The strain under examination was grown for 24 hours on a slant containing medium 21. From this a culture in log phase was obtained by transferring 10^3 cells/ml into a tube of liquid medium 21 and growing overnight. From this culture a suspension of approximately 10^7 cells/ml was made. One tenth of a milliliter of a 1/10 dilution of that suspension was transferred to each of 15 tubes containing 10 ml of medium 21, thus making a concentration of 10^4 cells/ml. The tubes were shaken on a reciprocating shaker at 28°C, usually for 17 to 19 hours, so that a concentration of the order of 2 to 6 $\times 10^7$ cells/ml was obtained in each tube (plateauing of growth is reached at 3 to 6 $\times$ 10^8 cells/ml). For strain 5209, cultures were allowed to grow closer to the plateau because the mutant frequency was very low.

When the 15 tubes were inoculated, the original suspension was assayed for the number of revertants it contained. Approximately 5×10^7 cells were transferred to plates containing minimal medium and the experiment was carried to its end only if no more than one to four revertants were found. This demonstrated that the frequency of revertants in the original suspension was less than 10^{-7}, and therefore that the probability of having introduced a revertant in each tube while inoculating 10^5 cells is practically 0.01.

After 17 to 19 hours of growth, each culture tube was assayed as follows: (1) Cells were counted with a hemocytometer, (2) each culture was divided into two 5-ml aliquots, (3) after centrifugation and resuspension in water, one of the aliquots was divided and plated on ten petri dishes (0.5 ml per dish) of minimal medium, and (4) the other 5-ml aliquot was centrifuged and resuspended in 5 ml of medium 23 for sporulation. The plates containing minimal medium were scored for residual growth by direct cell counting after 48 hours and scored for frequency of revertants after five days.

Residual growth: The cells plated on petri dishes occur singly or in physically attached groups of two, three, or four with a few groups composed of more than four cells (Table 1). Eight hundred to 1000 groups were counted microscopically

TABLE 1

Method of estimation of the residual growth in each plate (see text)

Strain	Scoring time (hours)	1	2	3	4	5	No. of cells per group 6 7 8 9 No. of groups scored				10	11	12	13	Total no. of groups	Avg. no. cells per group	Residual growth index (b/a)
5207	0	299	415	58	44	11	4								831	1.87(a)	1.63
	48	123	325	312	100	86	35	19	14	7		1	6		1028	3.05(b)	

in every plate. It has been determined that residual growth is complete by 48 hours, so at this time another 800 to 1000 groups are scored. The ratio between the second and first score is the factor of increase in numbers of cells (the residual

growth index in Table 1), so that the actual frequency of revertants per cell grown in the same dish may be computed correctly. This control was done on at least four dishes in each experiment. Residual growth was always seen to be as constant as it was for the examples listed in Table 2.

TABLE 2

Examples of residual growth indexes in some of the strains tested (see text)

Strain	Reversion selected	Experiment	Plate	Residual growth index	
5207	$ar_8\ Ar_8$	I	1	1.63	
			2	1.47	
			3	1.92	
			4	1.45	
			5	1.70	
					$\bar{x} = 1.63$
5160	$met_2\ Met_2$	I	1	1.15	
			2	1.95	
			3	1.40	
			4	1.26	
					$\bar{x} = 1.44$
5160	$met_2\ Met_2$	II	1	1.40	
			2	1.12	
			3	1.63	
			4	1.32	
					$\bar{x} = 1.37$
10102	$hi_1\ Hi_1$	I	1	1.60	
			2	1.67	
			3	1.82	
			4	1.73	
					$\bar{x} = 1.71$

Mutation rate: From the frequency of revertants and the total number of cells per tube, the mutation frequency per cell per division (mutation rate) was calculated by the method of the median of LEA and COULSON (1949, pp. 277–279). This mutation rate was obtained by dividing the number of mutations per culture, m, by the average number of cells per culture, assuming that the majority of mutations occurred during the last cell generation, since, when the inoculum is negligible, the surviving number of cells dividing is half or slightly more of the total number of divisions that have occurred previously. Also using the method of the median, the cultures with high numbers of mutations (where mutations might have occurred early) would be eliminated from the analysis. In every experiment the theoretical standard error computed from expression (41) of LEA and COULSON was always in agreement with the experimental standard error. Experimental standard errors are given throughout.

Since, in most experiments, the cells are diploid, if one assumes that mutations occur at the same rate in each chromosome, the mutations per diploid cell per division given throughout must be divided by two to obtain the spontaneous mutation rate in terms of mutations per chromosome per nuclear division.

Meiosis

After 48–72 hours in sporulation medium, cells from each tube were washed and treated with BPE for 24 hours to dissolve the ascus walls. This suspension of spores and unsporulated cells (the latter were never more than ten to 20 percent) was plated, after washing, in ten dishes of minimal medium. Countings for residual growth were unnecessary because previous tests performed on all our strains had shown clearly that spores do not germinate and unsporulated cells never bud when they are plated on minimal medium after being in sporulation medium.

Plates were scored after five days. The frequency of reversion was computed on the basis of the hemocytometer counts of the number of inoculated cells (spores and unsporulated cells.) Control experiments showed that, under these conditions, survival of both spores and unsporulated cells is always more than 95 percent. The revertant frequency per spore was computed after correcting the absolute revertant number by subtracting a percentage of revertants equal to the percentage of diploid clones among the revertants. This was done since these diploids were undoubtedly the progeny of unsporulated cells.

Some experiments were made for testing the magnitude of the error which might thus arise. In a mutation test on a sporulated culture different types of cells are plated on minimal medium (i.e., unsporulated cells, single spores and a few spores attached to each other pairwise). The unsporulated cells give rise to a pure diploid colony, the single spores to a haploid colony, and the double spores to either haploid or diploid or mixed colonies depending on the mating type of the two sister spores. The proportion of haploid revertant colonies was tested at least once for every type of mutant and it was found to be slightly lower than that expected from the frequency of single spores plated.

This can be due to two different phenomena:

1. The mutation frequency during meiosis is higher in those cells which do not accomplish meiosis (unsporulated cells) although they certainly reach some stage of the meiotic process as demonstrated by segregation of genetic markers.

2. Revertants from single spores produce a small haploid colony which, during its growth, encounters some nonreverted resting spores of opposite mating type (the average distances between individual spores in our dishes is not more than 50–100 microns) and copulation occurs on agar giving rise to a mixed haploid-diploid colony which in our test (sporulation test) was scored as diploid.

In the few cases where the discrepancy between frequency of haploids among revertants and the frequency of haploid spores in the inoculum seemed excessive, an analysis for appropriate genetic markers of the apparently diploid revertant colonies showed that they were mixed often enough to make hypothesis 2 a satisfactory explanation. Therefore, the procedure for estimating revertants among spores seems in order.

Revertants observed after sporulation can have different origins: (1) the reversion occurred in the mitotic cycle before sporulation and (2) the reversion occurred during the meiotic process. A distinction between the two origins is a

prerequisite to computing the mutation rate in meiosis. It is necessary, therefore, to know whether the revertant vegetative cells are homozygous or heterozygous for the selected mutation. It could be assumed that they are practically all heterozygous because the coincident occurrence of two independent mutational events in both chromosomes of the same cell is too low for affecting the experimental estimate of the revertant frequency. Nevertheless, it was considered worthwhile to test this assumption.

On three independent occasions, twice with strain 5160, and once with strain 5207, 50 vegetative revertants were taken at random from the minimal medium, individually sporulated, and four asci per revertant were dissected. Out of 150 revertants analyzed, only one from strain 5207 was a homozygous prototroph. Analysis of other markers carried by the strain demonstrated that coincident somatic recombination had occurred for the ur_3 and hi_3 loci. So, we concluded that homozygosity for the reversion was probably caused by somatic recombination rather than by two independent mutations. Anyhow, this experiment showed conclusively that within a probability of one percent or less, all revertants are heterozygous for the selected mutation.

On this basis, it is easy to estimate the contribution of revertants occurring during vegetative growth to the frequency of revertants among spores. Every vegetative revertant will produce, on the average, two revertant and two nonrevertant spores. *Therefore, one half the frequency of revertants in vegetative cells is subtracted from the corresponding frequency among spores to obtain an accurate estimate of the number of mutants produced during the two meiotic divisions.*

Every mutational event occurring during one meiotic cycle either in the first or second meiotic division gives rise to one revertant and three nonrevertant spores. Therefore the mutation rate (mutants per nucleus per division) will be equal to four times the frequency of revertants observed among the spore population if it is assumed that meiosis is a one-division process. If both first and second meiotic divisions are considered of importance, then the mutation rate will be lower. We are aware that spontaneous mutations could possibly occur at the time of DNA duplication, which would make a one-division process the interpretation of choice. However, in the absence of direct evidence for the time of occurrence of the observed spontaneous mutations, we have adopted the criterion that meiosis is a two-division process since it sets a lower limit for meiotic mutation rates. *Therefore, the corrected number of revertants per spore was multiplied by two and taken as the spontaneous rate for meiosis.* Whatever factor is used to obtain the mutation rate for the meiotic process, however, the observed mutation rates for mitosis and meiosis remain significantly different, as will be seen in the results.

The rates of reversion from auxotrophy to prototrophy for three different loci independent sporulations for each experiment, and the standard error has been calculated by the standard statistical procedure.

RESULTS

The rates of reversion from auxotrophy to prototrophy for three different loci

in three different strains are shown in Table 3. It is clear that the rate of spontaneous mutation during meiosis is from six to 20 times higher than that observed in mitosis.

TABLE 3

Mutation rates ($\times 10^8$) for replicate experiments of three different biochemical markers (reversion from dependence to independence)

Strain	Mutant	Mitosis	Meiosis
5160	met_2	4.86 ± 1.01	29.6 ± 3.12
		3.42 ± 0.52	58.0 ± 6.16
		3.93 ± 0.82*	26.8 ± 10.86*
		5.91 ± 0.76	40.4 ± 5.92
		6.53 ± 0.99	45.4 ± 7.62
5207	ar_8	5.90 ± 1.12	132.6 ± 10.82
		5.03 ± 0.87	43.2 ± 4.62
5209	hi_1	0.21 ± 0.031	2.2 ± 0.18
		0.28 ± 0.057	1.42 ± 0.17

* Significantly different at five percent level by t-test. All other data are significantly different below one percent level.

One obvious environmental difference between vegetative and sporulating cells is the presence of sodium acetate in the latter.

Two kinds of experiments proved that sodium acetate is not endowed with any mutagenic action, at least on our material. Yeast cells were grown mitotically in vegetative medium and also in the same medium with 0.5 and one percent of sodium acetate added. In the three media the mutation rate remained unchanged. The same vegetative culture was transferred for sporulation in acetate medium 23 and in tap water; sporulation occurred at the same frequency in the two although more slowly in water. Under the two different conditions, the meiotic mutation rate remained unchanged.

A mutagenic action of sodium acetate being excluded, several alternative possibilities formulated to account for the difference in mitotic and meiotic mutation rates were examined experimentally.

Selection for prototrophs: The first possibility was that of selection for the revertant prototroph in the sporulation medium. The following experiment was done to check whether both the auxotroph and the prototroph can grow in the sporulation medium prior to sporulation.

From a presporulation culture of strain 5160 (methionine-requiring) approximately 10^7 cells/ml were inoculated into sporulation medium 23 with and without 25 μg/ml of methionine. The variation in cell number was determined every four hours by hemocytometric readings. The results are shown in Figure 1a. It appears that in the absence of methionine no growth occurs, while in the presence of the amino acid cell number increases about 20 percent.

The same experiment was repeated using as an inoculum a mixture of ten revertant strains. The results are shown in Figure 1b. With or without methionine, cell number increases about 20 percent.
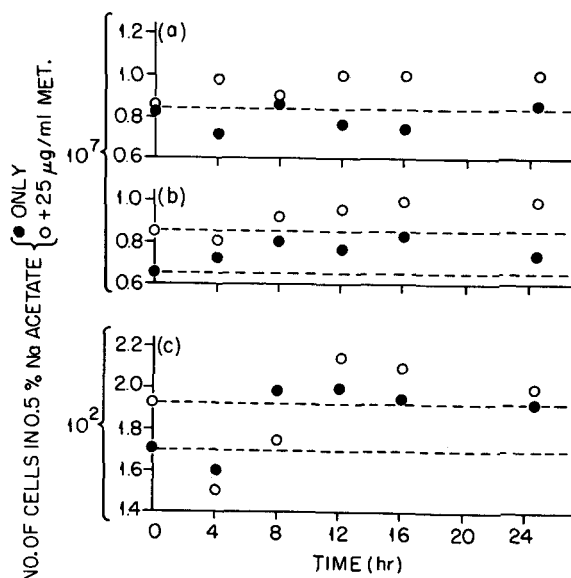
FIGURE 1.—Experiments to show the growth (number of cells/unit time) of the revertant prototroph in 0.5 percent sodium acetate sporulation medium, ●; and in 0.5 percent sodium acetate medium + 25 μg/ml methionine, ○: (a) met_2 cells, (b) mixture of ten revertant strains of Met_2 cells, (c) mixture of ten revertant strains of Met_2 cells in the presence of 10^7 met_2 cells.

To reconstruct the spontaneous mutation experiment as closely as possible, a small number of prototrophs (10^2 cells/ml) together with a large number of auxotrophs (10^7 cells/ml) were inoculated into the media. The growth of the prototrophs was tested in this experiment by plating on minimal medium. The results are shown in Figure 1c. Cell number of the prototroph increased approximately 20 percent, demonstrating that no more than an average of 0.2 divisions per cell could have occurred.

These data rule out the possibility of prototroph selection as the explanation for the observed difference in spontaneous mutation rates.

Nature of revertants: Another possible explanation is that different types of mutations were scored in the two systems—true back mutations (dominant) in the diploids (mitosis), and true back mutations plus recessive suppressors in haploids (meiosis). Dominant suppressors would not affect differently the estimates of mutation rate in the two systems.

This hypothesis has been tested by an experiment consisting of a spontaneous mutation rate analysis during mitosis in a haploid strain. Results are shown in Table 4. It can be seen that haploids mutate as expected, that is, at more or less one half the rate of diploids. Two different loci were tested in the same strain. These data show that recessive suppressors, if any, do not significantly affect the observed mutation rate when they are sought in haploid cells or spores. Suppressors would be exposed under these conditions even if they were intragenic.

TABLE 4

*Mutation rates ($\times 10^8$) for haploid and diploid cells
(reversion from dependence to independence)*

Strain	Ploidy	Mutant	Mitosis	Meiosis
5160	Diploid	met_2	4.09 ± 1.0*	40.6 ± 4.04
10102	Haploid	met_2	1.59 ± 0.097*	
5209	Diploid	hi_1	0.21 ± 0.031*	1.42 ± 0.17
10102	Haploid	hi_1	0.09 ± 0.010*	

* Observed diploid–haploid ratios are not significantly different from expected 2:1 ratios.

Differential sporulation: A further possibility is that revertant prototrophs sporulate better than the original auxotrophs. Even if this should hold true, the high frequency of sporulation of the auxotrophs makes certain that only a very small proportion of the increase in mutation rate during meiosis could be accounted for by differential sporulation. Further, a careful analysis of frequency of sporulation of 32 revertants indicated that their sporulation frequencies were not significantly different from parental auxotrophs.

Generation time: Another possibility is implicit in the very different rates of reproduction of meiotic and mitotic nuclei. Since the rate of meiosis cannot be easily changed (sporulation is severely reduced), we grew vegetative cells under identical growth conditions (reciprocating shaker, 28°C, etc.) in different media. The results are shown in Figure 2. The reproduction rate was decreased when glucose was replaced with glycerol in the medium or when the antifungal agent trichomycin was added to glucose medium. With trichomycin the generation time was decreased sixfold.

The spontaneous mutation rates obtained when diploid vegetative cells were grown on glycerol or trichomycin media are listed in Table 5. Mutation rates are time independent under the conditions of the experiment.

TABLE 5

*Mutation rates ($\times 10^8$) for cells with different generation times
(reversion from dependence to independence)*

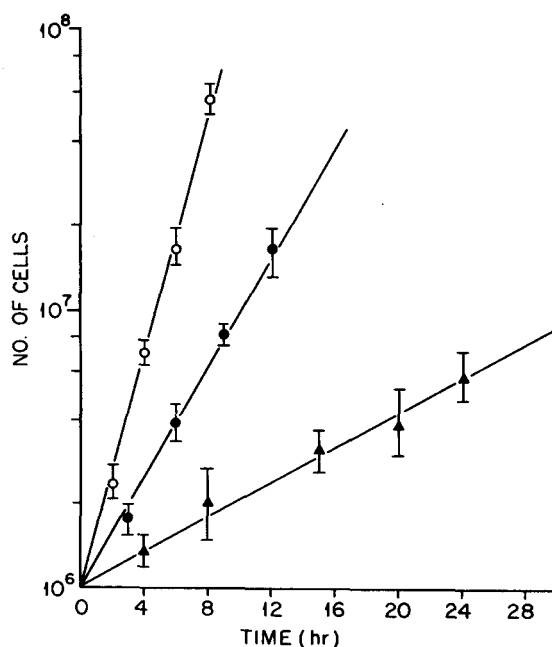| Strain	Mutant	Vegetative growth (mitosis)		Mutation rates	
		Culture medium	Generation time (in hr and min)	Mitosis	Meiosis
5160	met_2	Glucose	1:24	5.91 ± 0.76	40.4 ± 5.92
		Glycerol	3:05	5.60 ± 0.51	
		Trichomycin	9:12	4.33 ± 0.98	
5160	met_2	Glucose	1:25	6.53 ± 0.99	45.4 ± 7.62
		Glycerol	2:40	2.88 ± 0.70	
		Trichomycin	12:40	4.13 ± 1.01	

FIGURE 2.—Growth rate of yeast cells. (a) In the presence of glucose, O, $g = 1$ hr 24 min; (b) with glycerol replacing glucose, ●, $g = 3$ hr 5 min; (c) with trichomycin added to the glucose medium, ▲, $g = 9$ hr 12 min.

This conclusion is contrary to NOVICK's and SZILARD's finding (1951), but our conditions are quite different from theirs. Also, though not necessarily in basic disagreement with NOVICK and SZILARD, KUBITSCHEK (1960) and KUBITSCHEK and BENDIGKEIT (1961) showed that the nature of the limiting factor during growth in the chemostat has an effect on the time dependence of mutation rates.

<div align="center">CONCLUSION</div>

The spontaneous mutation rate (reversions per nucleus per division) of cells of *Saccharomyces cerevisiae* undergoing meiosis is six to 20 times that of cells mitotically reproducing for three different mutations tested.

<div align="center">SUMMARY</div>

The spontaneous mutation rate (reversions from dependence per nucleus per division) has been determined in diploid strains of *Saccharomyces cerevisiae* homozygous for three biochemical requirements (methionine$_2$, histidine$_1$, and arginine$_8$). The rates were estimated during vegetative reproduction (mitosis) and sporulation (meiosis). It was found that the mutation rate in cells undergoing meiosis is six to 20 times that of cells undergoing mitosis. The difference is statistically significant.

Possibilities for artifacts were analyzed, those associated with (1) prototroph selection during meiosis, (2) different nature of scored revertants, (3) differential sporulation frequency of revertants and auxotrophs, and (4) difference in generation time of mitotic and meiotic reproduction. No one of the possible artifacts can account for even a small proportion of the observed differences.

ACKNOWLEDGMENT

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